Pharmacogenetics of osteoporosis-related bone fractures: moving towards the harmonization and validation of polymorphism diagnostic tools

Osteoporosis is one of the most common skeletal chronic conditions in developed countries, hip fracture being one of its major healthcare outcomes. There is considerable variation in the implementation of current pharmacological treatment and prevention, despite consistent recommendations and guidelines. Many studies have reported conflicting findings of genetic associations with bone density and turnover that might predict fracture risk. Moreover, it is not clear whether genetic differences exist in relation to the morbidity and efficiency of the pharmacotherapy treatments. Clinical response, including beneficial and adverse events associated with osteoporosis treatments, is highly variable among individuals. In this context, the present article intends to summarize putative candidate genes and genome-wide association studies that have been related with BMD and fracture risk, and to draw the attention to the need for pharmacogenetic methodology that could be applicable in clinical translational research after an adequate validation process. This article mainly compiles analysis of important polymorphisms in osteoporosis documented previously, and it describes the simple molecular biology tools for routine genotype acquisition. Validation of methods for the easy, fast and accessible identification of SNPs is necessary for evolving pharmacogenetic diagnostic tools in order to contribute to the discovery of clinically relevant genetic variation with an impact on osteoporosis and its personalized treatment.

KEYWORDS: antiresorptives ■ BMD ■ fracture ■ harmonization ■ osteoporosis ■ pharmacogenetics ■ validation

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Osteoporosis is a systemic skeletal disease characterized by low bone mass and deterioration in the microstructure of bone tissue, which causes bone fragility and consequent increase in fracture risk [1].

Hip fracture (HF) is the most threatening osteoporotic fracture (OF) because of its high mortality rate that can reach 30% the year after the fracture [2], and 40% in the second year after fracture [3]. In addition, 40% of individuals who survive do not regain their previous health status and become dependent on others to be able to perform daily activities [201].

One of the most used and best clinical determinants of bone status of an individual, as well as OF, is evaluated through the measurement of BMD [4].

Although, many external factors play fundamental roles in determining BMD, it has been estimated that over 50% of women and 70% of men who have suffered fractures did not have previously taken osteoporotic BMD values [5]. Furthermore, in studies of osteoporosis therapy, increases in BMD were not linearly proportional to fracture risk reductions. The change in BMD induced by antiresorptive drugs explains only approximately 15% of the reduction in fracture risk [6].

Despite OF generally being a direct consequence of bone fragility, and therefore a key component of osteoporosis phenotype, OFs can occur as a result of high bone turnover and/or nonskeletal factors, such as the tendency to fall [7].

Genetic studies of osteoporosis have focused on BMD as the most influential predictor of fracture risk [202]. These studies were triggered by evidence that bone characteristics have proven to be highly heritable in twins and families, with 60–80% of variance being attributable to heritable factors [7].

Since osteoporosis has a complex and variable phenotype, and because of its epidemiologic interest, many related causative factors have been sought. These have included effort to establish an association between specific responsible genes or gene groups whose effects could interact together [8].

Thus, large numbers of studies have been reported over the past 15 years regarding osteoporosis genes. However, conflicting results have been obtained, specifically in those studies that examined the impact of polymorphic molecular structures. It is possible that this is owing to small sample sizes and lack of statistical power. Recently, huge efforts have led to the application of high-throughput methodology

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(i.e., genome-wide association studies [GWAS]) to elucidate relevant polymorphisms associated with clinical data (briefly summarized later). The information obtained from these studies is much appreciated, but its validity has to be confirmed and replicated for each population. Moreover, it is important to consider genetic data with several potential interaction factors and environmental factors, such as diet, ethnicity and lifestyle. All of these effects are well integrated in a recent GWAS of vitamin D levels (VDR) levels and polymorphisms that present an assessment with genotyped data replication [9].

Validation of functional polymorphic variants across different populations is a task for the near future that should not be restricted exclusively to large sampling or expression-profiling research [10].

As rapid technological advances provide increased accuracy and precision, issues related to validation must still be addressed. There is a need for the parallel validation of these technologies as they make the transition from research applications to routine clinical practice [11]. Technologies that can accurately identify genetic polymorphisms will dramatically affect routine diagnostic processes and future therapeutic developments in personalized medicine.

There are different priorities among countries to take into account, especially on the issue of funding for translational research. In many hospital settings, healthcare is focused on the treatment of existing disease and little or nothing is done to prevent the underlying disease. Therefore, economic analysis should be performed to examine the cost benefit analysis of the disease. However, the contribution of pharmacogenetics combined with pharmacoeconomics could benefit from studies regarding direct–indirect hospital spending on monitoring treatments performed *in situ*.

Taking these points into account, a significant clinical and scientific challenge will be the contribution towards developing and improving an accessible, rapid and easy methodology for the identification of SNPs, thereby, facilitating the evolution of pharmacogenetic diagnostic tools, and so cause a real impact in osteoporosis research worldwide.

In this context, the purpose of the present article is to describe the current state of the art techniques used in the study of factors affecting osteoporosis, specific pharmacotherapeutic treatments, selected putative candidate genes for BMD, osteoporosis and of risk, and their interindividual variability. Hopefully, these factors will be able to serve as models in the compliation and use of tools for genotype validation and replication studies. Consequently, this work intends to highlight the need for harmonizing SNP nomenclature and making simple molecular biology tools for establishing routine clinical research methodology for SNP determination easily available, such as PCR-RFLP, PCR-sequencing and/or allele discrimination methods.

Identification of individual fracture risk profiles

The assessment of fracture risk has been mainly based on estimation of BMD and past history of fractures [12,13]. However, even though BMD is a very potent risk factor, it cannot itself explain the whole risk presented for every patient. Several clinical guides [201], currently recommend treating women with a BMD T-score lower than -2 without risk factors, women with a T-score lower than -1.5 and one or more risk factors and women with a past history of vertebral and/or HF, irrespective of BMD. Nevertheless, fracture risk is directly related to BMD, even though there is no threshold value that predicts exactly who is going to suffer a fracture. Moreover, the efficacy rate of treatments for osteoporosis is only 48 % [14]. The individual likelihood of suffering a fracture depends on the combination of several risk factors [15–17]. Therefore, the prophylactic treatment for preventing fractures should be designed, at least in part, using the risk profile for each individual. Advances in genetics knowledge and environmental factors may allow a more personalized design for osteoporosis therapy and the prevention of fractures. Since there is an unpredictable response to different treatments, genetic information can, thus, potentially be used to identify patients who are likely to respond (or unlikely to respond) to a specific pharmacological therapy. Pharmacogenetic data can contribute to developing this insight.

Absolute estimation for the probability of major osteoporotic fracture & treatment initiation: fracture risk assessment tool

The WHO has proposed that drug therapy for treatment of osteoporosis should be considered when there is a BMD value between -2.5 or less in total for hip, femoral neck and/or lumbar spine BMD [18]. What is unclear, is who should receive pharmacologic treatment out of those patients who are at the stage of osteopenia (T-score between -1 and 2.5).

In order to resolve this discrepancy, the WHO have developed an algorithm called fracture risk assessment tool (FRAX®) that can calculate the probability of fracture over 10 years, providing specific risks for HF and OF. This algorithm uses both the femoral neck BMD (optional) and clinical risk factors with an impact on fracture risk independently of BMD [203]. Moreover, this tool is used only for postmenopausal women and men aged over 50 years, who have not previously received drug treatment.

This algorithm is freely available and allows physicians and patients to make more informed decisions based on the potential risk of fracture without treatment against the benefits and possible adverse effects of different therapeutic agents.

Although there is no universal consensus on the cutoff point to establish the drug treatment for a patient, in some countries such as the USA and UK, a FRAX rate for HF between 3 and 7%, respectively, has been proposed [19,20]. In Spain, this consensus has not yet been implemented and validated; however, an internal recommendation has been to initiate drug treatment when FRAX is 5% for HF (data not shown).

As well as FRAX, a 'Fracture Risk Calculator' tool has been developed and validated using data from the internationally renowned Dubbo Osteoporosis Epidemiology Study conducted by the Osteoporosis and Bone Biology Program of the Garvan Institute of Medical Research (Sydney, Australia). The study, which began in 1989, includes more than 2500 men and women aged 60 years or more from the Australian city of Dubbo. The longest running study of its kind in the world, it has contributed major changes to our understanding of osteoporosis in women and men, including fracture risk, impact on quality of life, and even survival [21,204].

Pharmacology treatment used for osteoporosis

Over the past two decades the range of therapeutic options available for the treatment of osteoporosis and fracture prevention has increased dramatically with the development of potent antiresorptive and anabolic agents. The antiresorptive agents include bisphosphonates (e.g., alendronate, risedronate, clodronate, etidronate, ibandronate and zoledronic acid), raloxifene, estrogen, and calcitonin. Anabolic agents include, for example, teriparatide (recombinant human parathyroid hormone 1–34) and possibly strontium ranelate, which have been suggested to induce a combination of modest effects on bone formation and resorption [22].

Bisphosphonates

Bisphosphonates are the most widely used class of currently approved inhibitors for bone turnover. Their main action is to inhibit bone resorption mediated by osteoclasts. Bisphosphonates bind to bone surface active sites and alter remodeling osteoclastic activity. It has been proposed that two distinct molecular mechanisms are responsible for the effects of these drugs on osteoclast function. Non-nitrogen containing bisphosphonates (e.g., etidronate and clodronate) alter the cell function to metabolize to analogs cytotoxic ATP-bisphosphonates. The nitrogen-containing bisphosphonates (e.g., alendronate, risedronate, ibandronate and zolendronic acid) have been shown to have greater antiresorptive power than non-nitrogen containing bisphosphonates [23]. The mechanism of action of this last group appears to be through its binding to farnesyl pyrophosphate synthetase, inhibiting enzymatic activity in the intracellular mevalonate (3-hydroxy-3-methylglutaryl CoA reductase) pathway and halting production of necessary sterols, cholesterols and lipids. Loss of these intracellular compounds leads to a decrease in post-translational modification of key proteins (Rab, Rac and Rho). Regulation of central osteoclast cellular activity is interrupted, and ultimately apoptosis of the osteoclast occurs. This mechanism of action seems to be limited to osteoclasts and is believed to be owing to the binding of the drug to hydroxyapatite sites in bone, with subsequent cellular ingestion occurring strictly with osteoclasts during bone resorption [24].

Raloxifene

Raloxifene, a selective estrogen receptor (ER) modulator, competitively inhibits 17-β estradiol at the ER. Binding of raloxifene to the ER results in a change in the receptor itself and prevents coregulator protein binding and activation of the blocked ER [25]. Raloxifene blocks activation of growth factor β-3, increasing the rate of osteoclast apoptosis and osteoblast differentiation, decreasing bone resorption and regulating bone remodeling [26].

Hormone replacement therapy

Hormone replacement therapy (HRT) such as 17-β estradiol (estrogen) acts on the bone in two-ways: directly and indirectly. The indirect action of estrogen involves the regulation of growth factors and cytokine production in osteoblasts which, in turn, regulate osteoclast differentiation and activity. Estrogens may also act directly on osteoclastic bone resorption [27].

Calcitonin

Calcitonin is an endogenous hormonal peptide produced in the thyroid. The release of calcitonin is increased by the elevation of blood calcium levels. It has been shown *in vitro* that, at low concentrations, calcitonin causes a rapid, albeit transient, change in the cytoskeletal structure of active osteoclasts. This structural change renders the osteoclasts ineffective but does not cause apoptosis of the osteoclast cell. The change in structure leads to a reduction in bone resorption. It has been reported that the effects of calcitonin are more apparent in trabecular bone than in cortical bone, which is most likely owing to increased bone turnover at the trabecular bone [28].

Teriparatide

Teriparatide is a recombinant formulation of the N-terminal chain 34-amino acid fragment of parathyroid hormone. Teriparatide works similarly to the endogenous hormone, regulating calcium and phosphorus metabolism in the bones and kidneys. Teriparatide, given in intermittent doses, acts as a bone anabolic agent via stimulating osteoblast function, increasing gastrointestinal calcium absorption, and increasing renal tubular resorption of calcium. When a higher continuous dose is achieved, it stimulates osteoclast function. Hence, teriparatide can increase or decrease bone mass depending on the dose [22].

Strontium ranelate

Strontium ranelate has a combination of modest effects on both anabolic and antiresorptive activity, increasing collagen and noncollagen protein synthesis, enhancing preosteoblast differentiation, inhibiting osteoclast differentiation and reducing osteoclast function [29].

New targeted therapy

Another new drug is denosumab [205]. It is a fully human monoclonal antibody to RANKL, an essential mediator of osteoclastic bone resorption [30]. RANKL plays a major role in the pathogenesis of postmenopausal osteoporosis, structural damage in rheumatoid arthritis, and bone loss associated with other skeletal disorders. In clinical trials performed in postmenopausal women with low BMD, denosumab increased BMD and suppressed bone resorption in a rapid, sustained and reversible manner. In women with postmenopausal osteoporosis, denosumab 60 mg subcutaneously given every 6 months reduced the risk of vertebral, hip and nonvertebral fractures compared with placebo. Compared with alendronate, denosumab provides a greater increase in BMD and greater decrease in bone turnover markers. Patients switching from alendronate to denosumab increase BMD more than those continuing alendronate [31]. There are, however, no head-to-head fracture end point comparison studies.

General pathways involved in bone homeostasis & main genetic factors

The recognition that several aspects of bone homeostasis are largely determined by genetic factors has led to an intensive search for specific genes associated with these quantitative and qualitative characteristics of bone and OF risk. In each case the first steps were to identify and verify a relationship between a gene and BMD [32]. Currently, many candidate genes have been investigated as valuable tools for their association with BMD and OF risk. However, the excitement surrounding early studies of allelic variation have often continued into controversy owing to the failure of independent replication, possibly owing to insufficient statistical power and false-positive results [33,34]. Genes involved in common pathways have been described as being related to the risk of osteoporosis, risk of hip and vertebral fractures and BMD values. These gene variants could affect homeostasis and bone structure, and thus measured BMD values.

Given the available studies from different reviews and given the clinical importance of osteoporosis, for this study we choose to be focus on those pathways and genes most studied in relation to BMD and osteoporosis fracture risk.

In this context, one of the active pathways is Wnt signaling that participates strongly in the process of bone formation and resorption, which include transmembrane proteins, and the LRP5 and LRP6 [35]. Other transmembrane proteins, the ITGB3, are vital for the maturation of osteoclasts and thus for bone resorption [36].

The osteoclastogenesis pathway is also involved in the process of bone remodeling, through the activity of OPG, RANK and RANK-L [37]. The two latter players in this signaling pathway enhance osteoclast number, survival and activity, while OPG acts as a competitor for binding to the RANKL receptor and thus inhibiting their activities [37,38].

Likewise, the active metabolite of vitamin D (1α,25(OH)2D3) plays a fundamental role in bone metabolism by its binding to its receptor, the VDR. It regulates calcium homeostasis through the binding and nuclear translocation of the 1α,25[OH]2D3 to regulate bone turnover and increase gut calcium absorption [39].

Collagen is an important component of the body's structural proteins. COL1A1 is the largest and most abundant constituent of all bone tissue proteins, and mutations in its structure or regulation are associated with osteoporosis [40].

Synthesis of estrogens is essential for the acquisition and maintenance of bone mass, predominantly in women [41]. The physiological functions of estrogens are performed when they bind to the α- and β-receptors (ESR-α, ESR-β), the final biological impact being expressed in both osteoblasts and osteoclasts [42].

Finally, FDPS, which is a key enzyme in the mevalonate pathway, has been demonstrated to be involved in the regulation of mechanisms by which bisphosphonates induce apoptosis of osteoclasts [43].

A summary of each of the genes with their respective signaling pathways is detailed in Table 1.

SNPs in potential candidate genes and genome-wide association studies related with osteoporosis, BMD & osteoporotic fractures

The search for and discovery of genes that are involved in the regulation of a clinical trait is mainly based on linkage or association, or both. Linkage analysis tracks the inheritance of a trait and identifies chromosomal regions that deviate from independent segregation with that trait. As previously described, association analysis determines whether the genetic make-up in those with and without the trait is different and seeks to identify specific DNA loci (or gene variants) that are responsible for the difference [44]. Linkage and association analysis use two major approaches for their gene search: GWAS and the screening of candidate genes [45].

The search for candidate genes is based on prior knowledge of the potential function of genes involved in metabolic pathways, including the biochemistry, pharmacology and physiology of bone formation and resorption. On the other hand, through genome-wide scanning, a set of markers on a genome map have been selected on the basis of utility without any *a priori* hypothesis (so-called hypothesis-free research) for analysis of association with a phenotype [46,47].

Moreover, recent reviews regarding genetic susceptibility to osteoporosis have compiled many candidate genes, which have been identified through studies of rare bone diseases, or through modern technologies such developing GWAS [48,49]. However, even these GWAS-identified loci require functional validation by replication studies and expression profiling relevant to skeletal biology systems [50].

Even though most genes of osteoporosis and their variants remain to be discovered, data on those genetic alterations that have already been demonstrated to have a clinical impact should be available for physicians. In this regard, while performing this study, the need for consensus in different pharmacogenetic aspects has become obvious. The current lack of consistency in SNP nomenclature necessitates a set of harmonization rules, accredited laboratories and guidelines from pharmacogenetic reference committees that would ideally provide global requirements for the validation of functional diagnostic SNPs affecting osteoporosis.

Current high-throughput methodologies such as GWAS have been performed on osteoporosis patients for finding and identifying the most important and common gene variants associated with osteoporosis and fracture risk [51–53]. Most of the SNPs associated with this disorder have been demonstrated via significant p-values in different studies [54] and different populations, and most of the common important SNPs found have been objects of careful analysis, and have been included in this article (see later and Table 1). In the near future, it is hoped that these results could be expanded to other genes and pathways (with further replication cohorts), along with a further examination of many SNPs potentially associated with BMD/osteoporosis, identified with statistical p-values just below

Table 1. Pathways and some of the candidate genes involved in osteoporosis and osteoporosis fracture.

Name of pathway	Putative candidate genes
Osteoclastogenesis	*OPG*, *RANK*, *RANK-L*
Wnt signaling	*LRP5*, *LRP6*, *ITGB3*, *ALOX-15*
Vitamin D	*VDR*
Estrogen	*ESR-α*, *ESR-β*
Collagen	*COL1A1*
Mevalonate	*FDPS*
Homeostasis calcium–phosphorus	*CaSR*

the cutoff values required for significance at the genome-wide level [55]. However, this powerful approach is not readily translated into routine hospital care, as it is used primarily for hypothesis testing rather than diagnosis and, in any case, the required skills and tools are not widely available. Nevertheless, these studies provide valuable data for approaching specific significant genotyping in osteoporosis and OF. The present work highlights some of the possible candidate genes belonging to specific pathways and their polymorphisms that have been already associated with genetic osteoporosis variability, BMD and/or osteoporosis fractures.

Thus, in the osteoclastogenesis pathway, a population study performed on 5861 men and women from Iceland, Denmark and Australia, identified SNPs linked to the values of BMD at the hip and spine level. SNPs in the genes for *OPG*, *RANK* and *RANK-L* were all associated with a variation of hip and spine BMD [56]. Furthermore, in a recent meta-analysis, a GWAS of 150 genes confirmed that three of these genes, *OPG*, *RANK*, *RANK-L*, reside in the same biological pathway, which influences bone resorption [57]. In another GWAS where a total of 2653 males from eight European countries were genotyped, genetics variants in the OPG–RANK–RANK-L signaling pathway influenced variation in bone turnover and BMD [58].

A GWAS conducted on 2074 women in the UK concluded that the SNP rs4755801 (4887 +3491G>A) of the gene for OPG, that is, harboring allele A, is significantly associated with BMD measurement and predisposes individuals to osteoporosis and osteoporotic fracture such as HF [53]. In another similar case–control study in patients with osteoporosis, BMD at the femoral neck area and total hip was lower in individuals with the allelic variant G of SNP rs3102735 (A163G) of *OPG* [59]. Moreover, other authors observed a significant association of this SNP with HF and wrist fracture in postmenopausal Caucasian women [60]. Another study performed in 136 Slovenian postmenopausal women, the aim of which was to find SNPs in the *OPG* gene and their possible association with BMD, the authors detected that a particular genotype, GG, in the SNP rs2073618 (G1181C) had significantly lower lumbar spine BMD than subjects displaying G1181C [61]. A separate study assessed previous results, whose minor C allele frequency in the same SNP rs2073618 was associated with higher levels of both N-terminal P1NP and CTX-1 and lower lumbar spine BMD [58].

In two GWAS performed in women of European background the SNP rs3018362 of the *RANK* gene was significantly associated with BMD in the hip area [56,62].

A study of 404 non-osteoporotic and osteoporotic postmenopausal Caucasian women, the SNP rs9533155 (G693C) of the *RANK-L* gene was associated with BMD of the femoral neck [63]. In a GWAS, as listed above, the SNP rs9594759 in RANK-L was significantly associated with lumbar BMD [56]. Also, in the study listed previously where 2653 men from eight European countries were genotyped, the minor allele of SNP rs9594759 (C) *RANK-L* was associated with lower P1NP, CTX-1 and BMD values [58].

In other studies of postmenopausal women, the SNP rs9525641 (C290T) in *RANK-L* was a risk factor for the susceptibility of postmenopausal osteoporosis at the femoral neck [64]. Other authors have also reported that the combination and expression alteration caused by variation on *OPG*, *RANK* and *RANK-L* genes could provoke gene–gene interactions that also influence BMD [65,66].

All these interesting results would be benefited and complemented by very new expression approaches linked to the existing SNPs that could increase the overall functional impact on BMD and fractures, similar to those recently described [50,67].

In the case of the Wnt/β-catenin pathway, several SNPs have been associated with variations in hip BMD [68]. In particular, in a randomized double-blind study in Caucasian men with osteoporosis the SNP rs3736228 (C3824T) of the gene *LRP5* was significantly associated with BMD in all of the areas within the proximal femur (i.e., femoral neck, total hip and trochanter) [69]. Similarly, in a study in young men not involved in any regular physical activity, individuals with the AA genotype of SNP rs4988321 (G1999A/C) had lower hip BMD [70]. In a prospective multicenter study in Europe and North America, among 37,534 individuals with prevalent fractures, lower femoral neck BMD and higher fracture risk was apparent in those with both alleles encoding Met667 and Val1330 of SNPs rs4988321 (G1999A/C) and rs3736228 (C3824T), respectively. Thus, this *LRP5* haplotype could be associated with HF in Caucasian [71].

In relation to the *LRP6* gene, in a study of 10,275 Dutch men and women, aged over 55 years, the SNP rs2302685 (G3184A) was associated with bone parameters of width and

height of the hip, and also with the risk of osteoporotic HF and vertebral fractures [72]. However, in another study no association of the polymorphism in *LRP6* was found for any osteoporosis phenotype [71].

In the case of integrins (e.g., ITGB3), in a study lasting 25 years of monitoring Danish men and women for HF risk, those homozygous for the SNP rs5918 (T176C) had double the risk of HF, mainly confined to postmenopausal women [73].

In other pathways, the *ALOX-15* gene, in women with the TT genotype of SNP rs7220870 (G48924T) in a longitudinal cohort study conducted in 9704 individuals from a white American population, had a higher rate of HF. Also, this SNP was associated with BMD change and the risk of other OF as well as HF [74]. However, a study in postmenopausal women from northeast London, UK, found no SNPs in *ALOX-15* to be significantly associated with the phenotype of BMD or fractures [75].

Currently, the pathway of vitamin D, is an important and very well studied pathway affecting bone and calcium homoeostasis. It involves the the *VDR* gene, which was the first gene candidate studied at molecular genetic association level [32]. Morrison *et al.* observed that the SNPs of the *VDR* gene, *Bsm*I rs1544410 (G1024 +283A), *Taq*I rs731236 (T1055C) and *Fok*I rs10735810 (T2C), were associated with variability in BMD of the femoral neck and trochanter in individuals. Also, in a study conducted in premenopausal women, postmenopausal women with or without osteoporosis and elderly men, all of them Caucasians, there was a significant relationship with *VDR* SNPs rs11568820, Cdx2 (G1739A), and the magnitude of BMD of femoral neck and total hip [65]. In addition, this same study found that in older men there was a significant association between *Fok*I rs10735810 SNP and the measurement of femoral neck BMD, and in the case of postmenopausal women, there was a significant relationship between femoral neck BMD and both SNPs (*Cdx2* rs11568820, *Fok*I rs10735810). Similarly, in postmenopausal women, there was a significant association of the *Bsm*I SNP rs1544410 with the measurement of femoral neck BMD together with the SNP rs11568820 *Cdx*2. Furthermore, in a prospective study in 589 postmenopausal women, the *Bsm*I SNP rs1544410 was associated with increased risk of HF independent of BMD, bone turnover markers, hormone levels and age [76]. However, this suggested that the mechanisms by which *VDR* genotypes might influence bone strength are unclear. On the other hand, a multicenter study, GENOMOS, did not find any association between the *Bsm*I rs1544410, *Taq*I rs731236 and *Fok*I rs10735810 SNPs, and BMD or fractures. However, these authors reported an association between *Cdx2* rs11568820 and risk of vertebral fractures [77]. In the same way, a meta-analysis evaluated the genetics effect of the *Bsm*I and *Taq*I polymorphism on fracture risk in 13 studies (1632 fracture cases and 5323 controls). The researchers did not find evidence of any relationship between these polymorphisms and fracture risk with any genetic model [78]. In other work conducted in 677 postmenopausal Caucasian women, individuals with the CC genotype of the *Taq*I SNP rs731236 (T1055C) had an increased risk of HF, independent of BMD and age [79]. Conversely, it is worth noting recent studies on the association between *VDR* polymorphisms and other risk factors in fracture such as falls, balance and muscle power without direct effect on BMD or fracture. For example, in one of the studies carried out in two separate Scottish population cohorts in postmenopausal women, the authors investigated the association between *VDR* polymorphisms (*Bsm*I, *Taq*I, *Fok*I, *Cdx2* and *Apa*I) and reported falls. They found an association for *Bsm*I SNP with falls, balance and muscle power measurements in both cohorts, specifically carriers of the A allele [80]. In addition, in another similar study of *VDR* polymorphisms (*Bsm*I and *Fok*l) and falls in a group of older Italian adults 80 years or more, the GG genotype of the *Bsm*I gene was associated with a reduced rate of falls compared with AA genotype, whereas no effect on falls was shown for *Fok*l polymorphisms [81].

In the pathway of estrogens, in a meta-analysis of work conducted in eight European clinical centers, ESR-α was involved in susceptibility to fracture risk and the *Xba*I SNP rs9340799 (C351G) contributed to fracture risk by mechanisms independent of BMD. This is despite BMD being a plausible biological mediator of the clinical effect for the polymorphisms involved in the estrogen pathway [82]. The authors also suggested that these effects could be mediated through effects on bone quality, geometry, turnover or other nonskeletal risk factors for fracture, such as muscle strength.

In a multicenter study on 641 premenopausal Caucasian women (aged 20–50 years), those with *Pvu*II SNP rs2234693 (T397C) in the ESR-α gene were more likely to report a family history of HF. Also the AA and AG genotype *Alu*I SNP rs4986938 (G1730A) in the *ESR*-β gene were strongly associated with this specific

type of fracture. It was also realized that those patients aged between 41 and 50 years with the AA genotype of the *Alu*I SNP rs4986938 (G1730A) in the *ESR*-β gene had a low bone density compared with the alternate homozygote, GG [83]. However, in a cohort study conducted in postmenopausal white women, no relationship was identified between the *Pvu*II SNP rs2234693 and *Xba*I SNP rs9340799 with hip BMD or risk of HF [74]. On the other hand, the effect of different polymorphic sites in the same gene were evaluated in a Italian population based study with respect to the three *ESR*-α gene polymorphisms combined (intron 1: *Pvu*II and *Xba*I; exon 1: TA dinucleotide repeat polymorphisms 5′ upstream) in 610 Italian postmenopausal women [84]. Becherini *et al.* observed strong linkage disequilibrium between intron-1 polymorphic sites and the microsatellite (TA)n dinucleotide SNPs, with a high degree of coincidence of the short TA allele and the presence of *Pvu*II and *Xba*I restriction site. They do not find any significant relationship between variability in intron-1 and BMD, but observed a correlation between (TA)n repeat allelic variants and lumbar BMD, where patients with a low number of repeats (TA <15) showed the lowest BMD values. They concluded that the (TA)n dinucleotide repeat polymorphism at the 5′ end of the *ESR*-α gene could account for part of the heritable component of BMD; however, no conclusive results were found in relation to fracture risk. A very similar study in a Danish population performed in 190 patients with vertebral fracture and 184 control patients found modest contribution of TA polymorphisms to the reduction of BMD and increased risk of OF [85]. Moreover, *Pvu*II, *Xba*I, *Bst*UI and TA repeat polymorphisms seems to be in linkage disequilibrium in both results studies [84,85].

Finally, in the collagen-1 pathway, the *COLA1 Sp*1 SNP, rs1800012 (G2046T) (referred to by Mann *et al.* authors as +G1245T) has been reported to be related with low BMD and the risk of HF. In a recent meta-analysis study there was a significant decrease in BMD values in patients with GT genotype and especially in those individuals homozygous for TT [86]. Similarly, another study found that those postmenopausal women with a homozygous TT genotype of the SNP, had increased risk of HF independent of BMD and age [79]. In the GENOMOS multicenter study, the *Sp*1 rs1800012 SNP was associated with femoral neck BMD with a recessive mode of inheritance [87]. In this regard, another case–control study in 462 Caucasian osteoporotic patients investigated the effect of the polymorphisms of collagen-1 and their haplotypes on the risk of osteoporosic vertebral fracture, BMD and biochemical markers of bone turnover. They found that the rs1800012 (G2046T) and rs24122298 (1663indelT) SNPs were in almost complete linkage disequilibrium. The T allele of the *Sp*1 rs1800012 (G2046T) and rs2412298 (1663indelT) SNPs were associated with lower lumbar spine BMD, whereas the T allele of the rs1107946 (G296T) SNP (referred to by Husted *et al.* as -1997 G/T) was associated with a minor effect on BMD, but increased the risk of vertebral fracture [88]. Moreover, in a large population-based cohort of an elderly Caucasian study, an increased risk of fragility fracture and lower BMD was observed in female carriers of the T allele in the *Sp*1 SNP. However, they did not find influence on fracture or BMD in postmenopausal women associated with the rs1107946 (G296T) polymorphism by itself, though power limitations cannot be excluded [89].

In summary, the results compiled for each pathway remain conflicting, possibly owing to the complexity of the osteoporosis phenotype itself, added to by limitations in the molecular tools available. These problems should be approached and resolved by common effort for developing and improving screening, risk assessment, diagnosis and treatment initiation [90]. In this sense, an important contribution would be the complementary use of validated genetic testing in clinical practice [91], specifically in pharmacogenetic analyses, which has been described in a recent compilation of genetic techniques [92].

Pharmacogenetics of the antiresorptive treatments for osteoporosis

Patient characteristics, presumably including genetic factors, determine individual responses for each specific drug [93].

Understanding the effects of such genetic factors not only offers the possibility of recognizing individuals that risk suffering an OF, but may also influence choice of antiosteoporotic drugs, whose clinical impact is measured by effects on BMD and markers of bone turnover.

Although, there is clear evidence of genetic influence on the variation in the efficacy and safety of treatment with pharmacological agents, there have been few conclusive data related to the pharmacogenetics of osteoporosis and OF,

and indeed, it is only recently that research on the genetics of osteoporosis has started in full swing [94].

The therapeutic breakthroughs that have emerged for treatment of osteoporosis may improve the quality and quantity of bone amongst a range of pharmacological alternatives (e.g., bisphosphonates, raloxifene, strontium ranelate, teriparatide and parathyroid hormone, among others), all of which are used for prevention of OF [95].

All these drugs have been shown to reduce the risk of OF to a greater or lesser extent, along with concomitant increases in bone density and decreases in high bone turnover [96]. In addition, clinical guidelines have recommended the use of bisphosphonates for both primary and secondary prevention of OF [206,207]. Moreover, it should be noted that all drugs used for osteoporosis have potential for adverse reactions that lead to concerns regarding their long-term use [22]. Bisphosphonates are predominantly used, because they are very effective drugs and particularly because of their falling cost with the availability of generic versions, clinical practice guidelines present them as priority drugs for the treatment of osteoporosis and/or prevention of fracture [206,207].

In many clinical trials, the relative risk reduction of fracture incidence is approximately 50%, showing a high variability in individual response to treatment [93]. In terms of BMD, even in well observed randomized controlled trials, it is estimated that 10% of patients do not respond as expected to antiosteoporotic therapy [97]. Thus, the efficacy and safety of bisphosphonates varies between individuals, with 5–10% being nonresponders and a small but significant proportion suffering clinical adverse events [98].

However, bisphosphonates are associated with adverse effects, including the arise of osteonecrosis of the jaw (ONJ). This has been reported most commonly in patients with bone metastases, its use is recommended in these patients owing to their high risk of bone pain and fractures [99]. In these cases, bisphosphonates are administered intravenously and usually at short intervals of weeks, rather than the annual dosing in osteoporosis, and often over years. The risk appears to be related to the overall dose of bisphosphonates used, but has also been associated with genetic polymorphisms. In one GWAS carried out in 2 groups of patients with multiple myeloma (22 with ONJ and 65 without), all receiving bisphosphonates over 2 years [100], researchers found that one SNP out of four in *CYP2C8* rs1934951, was associated with a risk of ONJ. Individuals homozygous for the T allele had increased likelihood of developing ONJ. This adverse reaction has also been linked to polymorphisms of *CYP3A* [208].

With regards to drug efficacy, several studies have examined the association of specific polymorphism with response to drug treatment of osteoporosis. Within the family of bisphosphonates, alendronate (ALN) has demonstrated efficacy in reducing bone resorption and reducing the risk of fragility fractures in postmenopausal women. Despite the overall effectiveness of ALN, there have been few studies evaluating the relationship between polymorphisms and response to this drug. One such study that was performed in osteoporotic postmenopausal women treated with ALN for 12 months, there was an association between *VDR* gene *Bsm*I SNPs and response to ALN, change from baseline in lumbar BMD being higher in GG than in AA *Bsm*I *VDR* genotype [101]. In another similar study [102], the *Bsm*I genotype was associated with effectiveness of ALN treatment, alone or in combination with other antiosteoporotics. For example, treatment with ALN and raloxifene led to a more marked improvement in BMD and bone turnover markers in patients with the homozygote GG or AA *VDR* genotypes. In heterozygous AG and homozygous GG patients, the combination of ALN with HRT and the association of raloxifene plus ALN had a stronger effect on BMD compared with either HRT or raloxifene treatment alone, but no more effectively than ALN alone.

Another of the few genetic studies on the effectiveness of ALN analyzed the relationship between the *ESR*-β gene *Rsa*I SNPs and treatment response of ALN in postmenopausal Caucasian women with osteoporosis. This group found no relationship between this SNP and changes of BMD or bone markers in response to ALN [103].

Another bisphosphonate, etidronate, has also been studied in relation to the *VDR* gene [104]. In these study 24 late osteoporosis postmenopausal women were evaluated during 1 year of treatment with etidronate and calcium supplementation. Lumbar spine BMD increased at a significantly faster rate in the AA and AG group compared with the GG group, and a significantly higher decrease in osteocalcin level was observed in the GG group as compared with AA subjects.

In the case of resorptive HRT, a US study assessed whether genotypes of *VDR* and *ER*, and their interaction, influenced changes in

bone mass in 108 European Caucasian postmenopausal women with and without HRT over 3 years [105]. In that study, the *VDR Bsm*I AA genotype was associated with larger spinal BMD increases using low HTR dose, whereas GG genotype was associated with larger BMD decrease in the placebo group. On the contrary, in a prospective randomized study of 429 healthy early postmenopausal Danish women with and without HRT over 5 years, no association was found between the BMD or change in BMD and either polymorphism studied (*Bsm*I and *Fok*I) [106].

Despite the contribution from these studies, it would appear logical to evaluate the efficacy of bisphosphonates at the genetic level in relation to their molecular mechanism of action. As discussed earlier, the nonamino-bisphosphonates are metabolized to ATP analogs and are accumulated in the cytosol of osteoclasts inducing cell death, while the amino-bisphosphonates inhibited prenylation of small GTPases that are essentials for osteoclast activity and survival (inhibiting enzymatic activity in the intracellular mevalonate) [107]. The effects of amino-bisphosphonates on the mevalonate pathway can explain not only their molecular mechanism of action, but also the different potency of the various amino-biphosphonate compounds. However, the role that the target enzymes of the mevalonate pathway play in the variability of the response to therapy with amino-bisphosphonates remains to be determined [108].

An example of this was reflected in a recent study in postmenopausal women, which evaluated the association between the SNP rs2297480 (A99C) in the *FDPS* gene (a key enzyme of the mevalonate pathway) and response after 2 years of treatment with bisphosphonates (ALN, oral and intravenous ibandronate). In that study, patients with CC genotype had a significantly poorer BMD response than the heterozygote or alternate homozygote [109].

Although these studies are preliminary, they strongly support the concept of pharmacogenetics as a powerful complement to studies of existing and novel drugs. These studies are a rich area for investigating truly relevant pharmacogenetic applications focused on bone-active metabolic pathways.

Molecular biology tools for 'in-house' routine genotyping of SNPs related to risk fracture & BMD

As noted earlier, a large number of genes have been investigated as possible markers for osteoporosis and fracture risk. However, there are no conclusive results to determine which are linked specifically to clinically relevant genetic variations. Also, the information available is not well harmonized, in terms of genetic nomenclature, such that this information is of low practical applicability for translational research, specifically in a hospital environment.

We have reviewed and screened the available information (see earlier) in order to describe in-house common genotyping tools that summarize some choices for analyzing specific SNPs that have been previously related with fractures and BMD. **Table 2** shows pathways, gene locus, code SNPs, gene/near gene, SNP locus, amino acid position, forward primer, reverse primer, applied technique, restriction enzyme, sequencing and a respective source of reference ID assays for TaqMan® (Applied Biosystems, CA, USA) genotyping for each polymorphism. We consider that this resource could simplify their use in clinical research. Also, we have summarized the SNP determination methodology that could be used as the routine tool of clinical research (PCR-RFLP, PCR-Sequencing and commercial allelic discrimination determination).

Thus, **Table 2** shows selected genes and SNPs from analyzed studies, representative of more common pharmacogenetic assays performed during the past 10 years. Given continuing controversy regarding the results of investigations into the genetics of osteoporosis [49] the main intention is to harmonize the existing data and to facilitate genotyping as part of translational clinical research, specifically for osteoporosis. Considerable work remains to be done in selecting the SNP according to the specific association being examined with respect to specific clinical issues. However, being aware of these advances will allow the more rapid implementation of new clinical genetic information in osteoporosis.

Pharmacogenetics has evolved according to the methodology available (allele specific amplification, PCR-RFLP, LightCycler® [Roche, Basel, Switzerland], Pyrosequencing [Biotage, Uppsala, Sweden], high-resolution melting curve analysis and microarrays techniques) [110]. There have been major technological changes from methods using unique SNP analysis of candidate genes to genome-wide analysis. However, the question remains as to how to optimise their use through study design. Moreover, the identification of a clinically relevant genetic variation in one single gene opens the way to examination of genes involved in linked pathways.

Table 2. Polymorphisms (SNPs) associated with putative candidates genes for BMD and osteoporotic fracture.

SNP description						Molecular biology tool					Ref.
Pathway/ gene	*Locus*	*SNP ID*	*Gene/ near gene*	*SNP locus*	*AA position change*	*Forward primer 5′–3′*	*Reverse primer 5′–3′*	*Method*	*RE*	*Assay ID†*	
OST	8q24	rs2073618	*OPG*	G1181C	Asn3Lys	ACTTCCTGTTGCCGG GACGCTA	ACTTCCTGTTGCCGGG ACGCTA	PCR/ RFLP	*Sml*I	C_1971047_1	[59]
	8q24	rs4355801	*OPG*	–	NA	ACATTTCAGGGGTTA TACTTGAC	CTGACTCTCTCTGACCT CCAC	PCR/Sq		C_8716062_10	TS
	8q24	rs3102735	*OPG*	A163G	NA	CCATCATCAAAGGG CTATTGGT	CTGGAGACATATAACT TGAACA	PCR/ RFLP	*Ase*I	C_1971046_10	[59]
	18q21	rs3018362	*RANK*	A>G	NA	GCTGAGGTGGGCTT GTATGTAGC	GGAGTCTAGGATGCTG AGGCAGC	PCR/Sq		C_15763310_10	TS
	13q14	rs9533155	*RANK-L*	G693C	NA	GAGGTCCAAGAGAT GGGTTTA	GAAGAGGTCAAAGAC TACAAGGACTA	PCR/ RFLP	*Bsa*JI	Not available	[64]
	13q14	rs9525641	*RANK-L*	C290T	NA	GAGGTCCAAGAGAT GGGTTTA	GAAGAGGTCAAAGAC TACAAGGACTA	PCR/ RFLP	*Mse*I	C_30171941_10	[64]
	13q14	rs9594759	*RANK-L*	–	NA	CCAGGCAAT TCAAA TACAT	GTGAGCAACCGCACC TTTC	PCR/Sq		C_44591336_10	[68]
Wnt	11q12-13	rs3736228	*LRP-5*	C3824T	Ala1330Val	GGGTCAGTGTGTGGA CCTG	GATGGGTGGAACTGC AGAGT	PCR/ RFLP	*Eci*I	C_25752205_10	[111]
	11q12-13	rs4988321	*LRP-5*	G1999A/C	Val667 Leu/Me	GGTGAGTCCTGAGCT CGGCACC	GTCTGAAGCCTTTGAG GCAGG	PCR/Sq		Not available	[69]
	12q13.2	rs2302685	*LRP-6*	G3184A	Val1062Ile	GCTGAGGTGGGCTTG TATGTAGC	CCGCTACATCTACTGGA CTTGTGAG	PCR/Sq		C_15757990_10	TS
	17q21.32	rs5918	*ITGB-3*	T176C	Leu33Pro	TTCTGATTGCTGGACTT CTCTT	CTCTCCCCACGGCAAAG AGT	PCR/ RFLP	*Msp*I	C_818008_30	[112]
	17p13.2	rs7220870	*ALOX-15*	G48924T	NA	CGGTGTCCTGGCGAGA GCAGGGAG	CCGTGGTACACACGTGC ATAACTC	PCR/Sq		Not available	TS
Vitamin D	12q13	rs1544410	*VDR*	G1024+283A	NA	GGCAACCTGAAGGGA GACGTA	CTCTTTGGACCTCATCAC CGAC	PCR/ RFLP	*Bsm*I	C_8716062_10	[65,113]
	12q13	rs7975232	*VDR*	A1025-49G	NA	CAGAGCATGGACAGG GAGCAAG	GCAACTCCTCATGGCTG AGGTCTCA	PCR/ RFLP	*Apa*I	C_28977635_10	[114]
	12q13	rs11568820	*VDR*	G1270A-G1739A	NA	CTGCAGCCTTGACCTC CTA	AAAGCAAACCAAGGGG TCTT	PCR/ RFLP	*Hyp*CH4II	C_2880808_10	[65]
	12q13	rs731236	*VDR*	T1055C	Ile352 Ile/Thr	AGAGCATGGACAGGG AGCAAG	GCAACTCCTCATGGCTGA GGTCTCA	PCR/ RFLP	*Taq*I	C_2404008_10	[65,115]
	12q13	rs10735810‡	*VDR*	T2C	Met1Thr	GCTGGCCCTGGCACTG ACTCTGCTCT	ATGGAAACACCTTGCTTC TTCTCCCTC	PCR/ RFLP	*Fok*I	Not available	[116]

†*Commercial assay.*
‡*Merged into rs2228570.*
§Fau*I can also be used.*
AA: Amino acid; NA: Not available; OST: Osteoclastogenesis; RE: Restriction enzyme; Sq: Sequencing; TS: This study.
The nomenclature is according to official HUGO Gene Nomenclature Committee [209].

Table 2. Polymorphisms (SNPs) associated with putative candidates genes for BMD and osteoporotic fracture (cont.).

SNP description					Molecular biology tool				Ref.
Pathway/ gene	*Locus*	*SNP ID*	*Gene/ near gene*	*SNP locus*	*AA position change*	*Forward primer 5′–3′*	*Reverse primer 5′–3′*	*Method*	*RE*	*Assay ID*[†]	
Estrogen	6q25	rs2234693	*ESR*-α	T397C	NA	GGGTTATGTGGCAATG ACG	GTTGCAGCAAAAGGTGT TGC	PCR/ RFLP	*Pvu*II	C_3163590_10	[117]
	6q25	rs9340799	*ESR*-α	C351G	NA	GGGTTATGTGGCAATG ACG	GTTGCAGCAAAAGGTGT TGC	PCR/ RFLP	*Xba*I	C_3163591_10	[117]
	14q23	rs4986938	*ESR*-β	G1730A	NA	GCTGGAGATGCTGAAT GCCCACGTGCTT	TCCTGACACACTGGAGTT CACGCTTCAGC	PCR/ RFLP	*Alu*I	C_11462726_10	[117]
Collagen	17q21	rs1800012	*COL1A1*	G2046	NA	TAACTTCTGGACTATTT GCGGACTTTTGG	GTCCAGCCCTCATCCTGGCC	PCR/ RFLP	*Sp*I	C_7477170_30	[118]
	17q21	rs1107946	*COL1A1*	G296T	NA	CACCCTGCCCTAGAC CAC	GAAAATATAGAGTTTC CAGAG	PCR/ RFLP	*Eco31*I	C__7477171_10	[119]
Mevalonate	1q22	rs2297480	*FDPS*	A99C	NA	ACAGATCTCAACCAGC GGG	TGATTAGTTGGGTCTCAG TCACC	PCR/ RFLP	*Smu*I[§]	C_2737970_10	[109]

[†]*Commercial assay.*
[‡]*Merged into rs228570.*
[§]*FauI can also be used.*
AA: Amino acid; NA: Not available; OST: Osteoclastogenesis; RE: Restriction enzyme; Sq: Sequencing; TS: This study.
The nomenclature is according to official HUGO Gene Nomenclature Committee [209].

As the studies progress, it will be important to recognize and adjust for the effect and contribution of ethnicity, environmental factors, diet, sunlight exposure, comedication, clinical status, clinical data availablility and lifestyle. Over the next few years, complex diseases such as osteoporosis will be treated with new therapies, and improved understanding of genetic variations that effect response to treatment and/or risk of side effects will force diagnostic companies to develop new tests that allow for the tailoring of a patient's treatment.

Conclusion

Progress in analyzing the human genome in combination with bioinformatic and technological advances have enabled better opportunities for understanding complex diseases with genetic determinants such as osteoporosis its fracture consequences. The successful identification of putative genes associated with fracture risk, variability and its pharmacogenetics must be approached in large studies combining relevant data from individual clinical data collections. Most of the larger studies have been made with genome-wide association data; however, it will be expected that practical large-scale studies should be replicated and validated in patients across different populations, in order to discover those genes that really contribute significantly to interindividual clinical variation of fracture phenotypes and of treatment responses.

Currently, despite most previous genetic osteoporosis studies having been focussed on SNPs analyses, there has been little or no harmonization of nomenclature. Hence, this article selectively summarizes emergent information that could be applied both now and as a model for future reporting of genetic information from fracture risk studies.

There are intrinsic difficulties in evaluating the impact of human genetic variability and linking this information to physiological mechanisms and to clinically significant effects. There are concerns as to whether there are real associations within different populations, which highlights the need for replication and confirmation data in large datasets, including different ethnicities. In this regard, genetic data determination as part of routine direct hospital testing could be a strategic key point for success in this complex task. The present work highlights not only the important contribution of high-throughput methodology such as microarray platforms, but the routine techniques available for all molecular biology research and clinical laboratories, which manage large numbers of patient samples and hence can contribute towards obtaining valuable genetic

and pharmacogenetic data for replication and their clinical interpretation and reproducibility in large-scale international studies.

At this time, the clinical challenge is to assess the validity and functionality of various SNPs with respect to osteoporosis risk. Clarification of these data are essential for convincing pharmaceutical and biotechnology companies to join with the development of commercial tests suitable for routine diagnosis in pharmacogenomics.

When this point is reached, the pharmacogenetic diagnostic information could help to reduce costs associated with outsourcing of laboratory services and, consequently, encourage hospitals that have adequate basic infrastructure to carry out their own pharmacogenetic analysis for better complementary drug monitoring assessment and contribute to better clinical outcomes in osteoporosis.

Future perspective

The main objective of pharmacogenetic research is to achieve confidence in the use of genetic tools for optimally selecting drugs for an individual and adjusting dose. This aim is currently hampered by limitations of global research. Well organized and controlled clinical trials are needed to demonstrate that pharmacogenetic interventions could really improve the pharmacotherapy. These clinical trials are developed under restricted hospital supervision, and thus the expansion of resources for pharmacogenetic tools would be a significant and contributive step towards patient benefit.

With the information compiled in this article, it would be possible to more easily use genotype–phenotype analyses to expand the data relating SNPs, in a wide range of relevant genes, to osteoporosis phenotypes including drug responses, in order to maximize the health and welfare of patients with osteoporosis and fracture risk. The genotyping of osteoporosis patients can be performed with basic molecular biology tools, and once specific genotype–phenotype associations are established or confirmed, these could be a very useful tool optimal treatments in every subject, avoiding suboptimal long-term treatment responses and/or adverse events.

The immediate challenge is to apply this methodology to completely ascertain and determine the extent to which these pathways or gene variants are present in our Caucasian population suffering OF versus a healthy control population and assess the degree of association that exists, as well as the extent to which they may influence therapeutic responses.

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Executive summary

- General inter-relation of pharmacogenetics knowledge and analyses of the risk fracture profiles of different treatments in relation to human genetic variability will lead the way towards personalized osteoporosis follow-up.
- Current knowledge of osteoporosis in genetics and pharmacogenetics has been obtained mainly through genome-wide association studies. However, there still are conflicting results and so there is a need for further functional validation assessment.
- Polymorphisms of *VDR*, *ESR*, *OPG/RANK/RANKL* and *COL1AI* have been identified as the more common markers to study for establishing an association between osteoporosis fracture risk and BMD.
- Harmonization of previous polymorphism nomenclature in important osteoporosis-related pathways will contribute to the facilitation of routine clinical studies for replication of results.
- New putative pharmacogenomics and pharmacogenetic biomarkers related to osteoporosis and its personalized treatment will be in constant evolution owing to the discovery of new genetic data, treatments and management of pathology.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy: Osteoporosis prevention, diagnosis, and therapy. *JAMA* 285(6), 785–795 (2001).

2 Franzo A, Francescutti C, Simon G: Risk factors correlated with post operative mortality for hip fracture surgery in the elderly: a population-based approach. *Eur. J. Epidemiol.* 20(12), 985–991 (2005).

3 Sosa M, Navarro R, Arbelo A: Fractura de cadera: la realidad española. In: *Actualización de osteoporosis.* Díaz Curiel M (Ed.). FHOEMO, Madrid, Spain, 13–22 (2001).

4 Kanis JA: Diagnosis of osteoporosis and assessment of fracture risk. *Lancet.* 359(9321), 1929–1936 (2002).

5 Nguyen ND, Eisman JA, Center JR, Nguyen TV: Risk factors for fracture in nonosteoporotic men and women. *J. Clin. Endocrinol. Metab.* 92(3), 955–962 (2007).

6 Delmas PD, Li Z, Cooper C: Relationship between changes in bone mineral density and fracture risk reduction with antiresorptive drugs: some issues with meta-analyses. *J. Bone Miner. Res.* 19(2), 330–327 (2004).

7 Nguyen TV, Howard GM, Kelly PJ *et al.*: Bone mass, lean mass, and fat mass: same genes or same environments? *Am. J. Epidemiol.* 147(1), 3–16 (1998).

8 Chen Y, Shen H, Yang F *et al.*: Choice of study phenotype in osteoporosis genetic research. *J. Bone Miner. Metab.* 27(2), 121–126 (2009).

9 Jiyoung A, Yu K, Stolzenberg-Solomon R *et al.*: Genome-wide association study of circulating vitamin-D levels. *Hum. Mol. Genet.* 19(13), 2739–2745 (2010).

10 Li WF, Hou SX, Yu B, Li MM, Férec C, Chen JM: Genetics of osteoporosis: accelerating pace in gene identification and validation. *Hum. Genet.* 127(3), 249-285 (2010).

11 Di Francia R, Frigeri F, Berretta M *et al.*: Decision criteria for rational selection of homogeneous genotyping platforms for pharmacogenomics testing in clinical diagnostics. *Clin. Chem. Lab. Med.* 48(4), 447–459 (2010).

▪ **Provides the reader with an overview of the various technologies used for pharmacogenomic testing in a routine clinical laboratory.**

12 Johnell O, Kanis JA, Oden A *et al.*: Predictive value of BMD for hip and other fractures. *J. Bone Miner. Res.* 20(7), 1185–1194 (2005).

13 Nguyen ND, Pongchaiyakul C, Center JR, Eisman JA, Nguyen TV: Identification of high-risk individuals for hip fracture: a 14-year prospective study. *J. Bone Miner. Res.* 20(11), 1921–1928 (2005).

14 Spear BB, Heath-Chiozzi M, Huff J: Clinical application of pharmacogenetics. *Trends Mol. Med.* 7(5), 201–204 (2001)

15 Cummings SR, Nevitt MC, Browner WS *et al.*: Risk factors for hip fracture in white women. Study of osteoporotic fractures research group. *N. Engl. J. Med.* 332(12), 767–773 (1995).

16 Rojo-Venegas K, Aznarte-Padial P, Calleja-Hernández MA *et al.*: Factors of risk in an elderly population: evaluation scales for the prevention of hip fractures. *Rev. Esp. Cir. Traumatol. Ortp.* 54(3), 167–173 (2010).

17 Kanis JA, McCloskey EV, Johansson H, Oden A: Approaches to the targeting of treatment for osteoporosis. *Nat. Rev. Rheumatol.* 5(8), 425–431 (2009).

18 Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. *Technical Report Series No. 843,* WHO, Geneva, Switzerland, 1–129 (1994).

19 Kanis JA, McCloskey EV, Johansson H, Strom O, Borgstrom F, Oden A: National Osteoporosis Guideline Group. Case finding for the management of osteoporosis with FRAX-assessment and intervention thresholds for the UK. *Osteoporos. Int.* 19(10), 1395–1408 (2008).

20 Tosteson A, Melton L, Dawson-Hughes B, *et al.*: Cost–effectiveness osteoporosis treatment thresholds: the United States perspective from the National Osteoporosis Foundation Guide Committee. *Osteoporos. Int.* 19(4), 437–447 (2008)

21 Nguyen ND, Frost SA, Center JR, Eisman JA, Nguyen TV: Development of prognostic nomograms for individualizing 5-year and 10-year fracture risks. *Osteoporos. Int.* 19(10), 1431–1444 (2008).

22 Gates BJ, Sonnett TE, DuVall CAK, Dobbins EK: Review of osteoporosis pharmacotherapy for geriatric patients. *Am. J. Geriatr. Pharmacother.* 7(6), 293–323 (2009).

23 Dunford JE, Thompson K, Coxon FP *et al.*: Structure-activity relationships for inhibition of farnesyl diphosphate synthase *in vitro* and inhibition of bone resorption *in vivo* by nitrogen-containing bisphosphonates. *J. Pharmacol. Exp. Ther.* 296(2), 235–242 (2001).

24 Matthew D, Clarke B, Khosla S: Bisphosphonates: mechanism of action and role in clinical practice. *Mayo Clin. Proc.* 83(9), 1032–1045 (2008).

25 Jensen EV, Jordan VC: The estrogen receptor: a model for molecular medicine. *Clin. Cancer Res.* 9(6), 1980–1989 (2003).

26 Yang NN, Venugopalan M, Hardikar S, Glasebrook A: Identification of an estrogen response element activated by metabolites of 17 β-estradiol and raloxifene. *Science* 273(5279), 1222–1225 (1996).

27 Saintier D, Burde MA, Rey JM, Maudelonde T, De Vernejoul MC, Cohen-Solal ME: 17b-estradiol downregulates b3-integrin expression in differentiating and mature human osteoclasts. *J. Cell Physiol.* 198(2), 269–276 (2004).

28 Carter PH, Schipani E: The roles of parathyroid hormone and calcitonin in bone remodeling: prospects for novel therapeutics. *Endocr. Metab. Immune Disord. Drug Targets* 6(1), 59–76 (2006).

29 Kendler DL: Strontium ranelate-data on vertebral and nonvertebral fracture efficacy and safety: mechanism of action. *Curr. Osteoporos. Rep.* 4(1), 34–39 (2006).

30 Bekker PJ, Holloway DL, Rasmussen AS *et al.*: A single-dose placebo controlled study of AMG 162, a fully human monoclonal antibody to RANKL, in postmenopausal women. *J. Bone Miner. Res.* 19(7), 1059–1066 (2004).

31 Lewiecki M: Denosumab update. *Curr. Opin Rheumatol.* 21(4), 369–373 (2009).

▪ **Denosumab is a promising therapeutic agent for the management of postmenopausal osteoporosis as it increases BMD more than alendronate.**

32 Morrison NA, Qi JC, Tokita A *et al.*: Prediction of bone density from vitamin-D receptor alleles. *Nature* 367(6460), 284–287 (1994).

33 Huang QY, Recker RR, Deng HW: Searching for osteoporosis genes in the post-genome era: progress and challenges. *Osteoporos. Int.* 14(9), 701–715 (2003).

34 Ioannidis JP: Why most published research findings are false? *PLOS Med.* 2(8), 696–701 (2005).

35 Tamai K, Semenov M, Kato Y *et al.*: LDL-receptor-related proteins in Wnt signal transduction. *Nature* 407(6803), 530–535 (2000).

36 Teitelbaum SL: Osteoclasts, integrins and osteoporosis. *J. Bone Miner. Metab.* 18(6), 344–349 (2000).

37 Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs L: The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. *J. Bone Miner. Res.* 15(1), 2–12 (2000).

38 Jones HD, Kong YY, Penninger JM: Role of RANKL and RANK in bone loss and arthritis. *Ann. Rheum. Dis.* 61(2), 32–39 (2002).

39 Wesley P, Hironori Y, Nirupama K: Vitamin-D receptor-mediated gene regulation mechanisms and current concepts of vitamin-D analog selectivity. *Adv. Ren. Replace. Ther.* 3(9), 168–174 (2002).

40 Byers PH: Brittle bones-fragile molecules: disorders of collagen gene structure and expression. *Trends Genet.* 6(9), 293–300 (1990).

41 Ichikawa S, Koller D, Peacock M *et al.*: Polymorphisms in the estrogen receptor β (*ESR2*) gene are associated with bone mineral density in Caucasian men and women. *J. Clin. Endocrinol. Metab.* 90(11), 5921–5927 (2005).

42 Bord S, Horner A, Beavan S, Compston J: Estrogen receptors α and β are differentially expressed in developing human bone. *J. Clin. Endocrinol. Metab.* 86(5), 2309–2314 (2001).

43 Bergstrom JD, Bostedor RG, Masarachia PJ, Reszka AA, Rodan G: Alendronate is a specific, nanomolar inhibitor of farnesyl diphosphate synthase. *Arch. Biochem. Biophys.* 373(1), 231–241 (2000).

44 Risch N, Merikangas K: The future of genetic studies of complex human diseases. *Science* 273(5281), 1516–1517 (1996).

45 Nguyen TV, Blangero J, Eisman JA: Genetic epidemiological approaches to the search for osteoporosis genes. *J. Bone Miner. Res.* 15(3), 392–401 (2000).

46 Dekker MC, van Duijn CM: Prospects of genetic epidemiology in the 21st Century. *Eur. J. Epidemiol.* 18(7), 607–616 (2003).

47 Rao DC: An overview of the genetic dissection of complex traits. *Adv. Genet.* 60, 3–34 (2008).

48 Ralston SH: Genetics of osteoporosis. *Ann. NY Acad. Sci.* 1192(1), 181–189 (2010).

49 Ralston SH, Uitterlinden AG: Genetics of osteoporosis. *Endocr. Rev.* doi:10.1210/er.2009-0044 (2010) (Epub ahead of print).

50 Hsu YH, Zillikens MC, Wilson SG *et al.*: An integration of genome-wide association study and gene expression profiling to prioritize the discovery of novel susceptibility loci for osteoporosis-related traits. *PLoS Genet.* 6(6), E1000977 (2010).

▪▪ **Efficiency of functional characterization using available expression profiling (relevant to the skeletal system) *in vivo* models to prioritize candidate genes for further functional validation.**

51 Styrkarsdottir U, Halldorsson BV, Gretarsdottir S *et al.*: Multiple genetic loci for bone mineral density and fracture. *N. Engl. J. Med.* 358(22), 2355–2365 (2008).

52 Nguyen TV, Center JR, Eisman JA: Pharmacogenetics of osteoporosis and the prospect of individualized prognosis and individualized therapy. *Curr. Opin. Endocrinol. Diabetes Obes.* 15(6), 481–488 (2008).

▪▪ **Identification of gene variants associated with response to therapy can eventually help individualize the prognosis, treatment and prevention of fractures and their adverse outcomes.**

53 Richards JB, Rivadeneira F, Inouye M *et al.*: Bone mineral density, osteoporosis, and osteoporotic fracture: a genome-wide association study. *Lancet* 371(9623), 1505–1512 (2008).

54 Rivadeneira F, Styrkársdottir U, Estrada K *et al.*: Genetic factors for osteoporosis (gefos) consortium. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. *Nat. Genet.* 41(11), 1199–1206 (2009).

55 Ferrari S: Human genetics of osteoporosis. *Best Pract. Res. Clin. Endocrinol. Metab.* 22(5), 723–735 (2008).

56 Styrkarsdottir U, Halldorsson BV, Gretarsdottir S *et al.*: Multiple genetic loci for bone mineral density and fracture. *N. Engl. J. Med.* 358(22), 2355–2365 (2008).

57 Richards JB, Kavvoura FK, Rivadeneira F *et al.*: Collaborative meta-analysis: associations of 150 gene with osteoporosis and osteoporosis fractures. *Ann. Intern. Med.* 151(8), 528–537 (2009).

▪▪ **Nine out of 150 candidate genes were associated with regulation of BMD, four of which also significantly affected fracture risk.**

58 Roshandel D, Holliday K, Pye SR *et al.*: Genetic variation in the *RANKL/RANK/OPG* signaling pathway is associated with bone turnover and bone mineral density in men. *J. Bone Miner. Res.* 25(8), 1830–1838 (2010).

59 Langdahl Bl, Carstens M, Stenkjaer L, Eriksen EF: Polymorphisms in the osteoprotegerin gene are associated with osteoporotic fractures. *J. Bone Miner. Res.* 17(7), 1245–1255 (2002).

60 Jorgensen HL, Kusk P, Madsen B, Fenger M, Lauritzen JB: Serum osteoprotegerin (OPG) and the A163G polymorphism in the *OPG* promoter region are related to peripheral measures of bone mass and fracture odds ratios. *J. Bone Miner. Metab.* 22(2), 132–138 (2004).

61 Arko B, Prezelj J, Kocijancic A, Komel R, Marc J: Association of the osteoprotegerin gene polymorphisms with bone mineral density in postmenopausal women. *Maturitas* 51(3), 270–279 (2005).

62 Styrkarsdottir U, Halldorsson BV, Gretarsdottir S *et al.*: New sequence variants associated with bone mineral density. *Nat. Genet.* 41(1), 15–17 (2009).

63 Mencej S, Albagha O, Prezelj J, Kocjan T, Marc J: Tumor necrosis factor superfamily member 11 gene promoter polymorphisms modulate promoter activity and influence bone mineral density in postmenopausal women with osteoporosis. *J. Mol. Endocrinol.* 40(6), 273–279 (2008).

64 Mencej S, Prezelj J, Kocijancic A, Ostanek B, Marc J: Association of *TNFSF11* gene promoter polymorphisms with bone mineral density in postmenopausal women. *Maturitas* 55(3), 219–226 (2006).

65 Mencej S, Prezelj J, Kocijancic A *et al.*: The combinations of polymorphisms in vitamin-D receptor, osteoprotegerin and tumor necrosis factor superfamily member 11 genes are associated with bone mineral density. *J. Mol. Endocrinol.* 42(3), 239–247 (2009).

66 Zupan J, Mencej-Bedrac S, Jurkovic-Mlakar S, Prezelj J, Marc J: Gene–gene interactions in RANK/RANKL/OPG system influence bone mineral density in postmenopausal women. *J. Steroid Biochem. Mol. Biol.* 118(1–2), 102–106 (2010).

▪▪ **Gene–gene interactions between *RANK* and *OPG*, and *RANK* and *RANKL* influence BMD in postmenopausal women.**

67 Chen XD, Xiao P, Lei SF *et al.*: Gene expression profiling in monocytes and SNP association suggest the importance of the *STAT1* gene for osteoporosis in both Chinese and Caucasians. *J. Bone Miner. Res.* 25(2), 339–355 (2010).

68 Sims AM, Shephard N, Carter K *et al.*: Genetic analyses in a sample of individuals with high or low BMD shows association with multiple Wnt pathway genes. *J. Bone Miner. Res.* 23(4), 499–506 (2008).

69 Kruk M, Ralston SH, Albagha OM: *LRP5* polymorphisms and response to risedronate treatment in osteoporotic men. *Calcif. Tissue Int.* 84(3), 171–179 (2009).

70 Brixen K, Beckers S, Peeters A *et al.*: Polymorphisms in the low-density lipoprotein receptor-related protein 5 (*LRP5*) gene are associated with peak bone mass in non-sedentary men: results from the odense androgen study. *Calcif. Tissue Int.* 81(6), 421–429 (2007).

71 van Meurs JB, Trikalinos TA, Ralston SH *et al.*: Large-scale analysis of association between *LRP5* and *LRP6* variants and osteoporosis. *JAMA*. 299(11), 1277–1290 (2008).

72 van Meurs JB, Rivadeneira F, Jhamai M *et al.*: Common genetic variation of the low-density lipoprotein receptor-related protein 5 and 6 genes determines fracture risk in elderly white men. *J. Bone Miner. Res.* 21(1), 141–150 (2006).

73 Tofteng CL, Bach-Mortensen P, Bojesen SE, Tybjaerg-Hansen A, Hyldstrup L, Nordestgaard BG: Integrin β-3 Leu33Pro polymorphism and risk of hip fracture: 25 years follow-up of 9233 adults from the general population. *Pharmacogenet. Genomics* 17(1), 85–91 (2007).

74 Tranah GJ, Taylor BC, Lui LY *et al.*: Genetic variation in candidate osteoporosis genes, bone mineral density, and fracture risk: the study of osteoporotic fractures. *Calcif. Tissue Int.* 83(3), 155–166 (2008).

75 Mullin BH, Spector TD, Curtis CC *et al.*: Polymorphisms in *ALOX12*, but not *ALOX15*, are significantly associated with BMD in postmenopausal women. *Calcif. Tissue Int.* 81(1), 10–17 (2007).

76 Garnero P, Muñoz F, Borel O, Sornay-Rendu E, Delmas PD: Vitamin-D receptor gene polymorphisms are associated with the risk of fractures in postmenopausal women, independently of bone mineral density. *J. Clin. Endocrinol. Metab.* 90(8), 4829–4835 (2005).

77 Uitterlinden AG, Ralston SH, Brandi ML *et al.*: The association between common vitamin-D receptor gene variations and osteoporosis: a participant-level meta-analysis. *Ann. Intern. Med.* 145(4), 302–304 (2006).

78 Fang Y, Rivadeneira F, van Meurs J, Pols HA, Ionnidis J, Uitterlinden A: Vitamin D receptor gene *Bsm*I and *Taq*I polymorphisms and fracture risk: a meta-analysis. *Bone* 39(4), 938–945 (2006).

79 Nguyen T, Esteban LM, White CP *et al.*: Contribution of the collagen I α1 and vitamin-D receptor genes to the risk of hip fracture in elderly women. *J. Clin. Endocrinol. Metab.* 90(12), 6575–6579 (2005).

80 Barr R, McDonald H, Stewart A *et al.*: Association between vitamin-D receptor gene polymorphisms, falls, balance and muscle power: results from two independent studies (APOSS and OPUS). *Osteoporos. Int.* 21(3), 457–466 (2010).

81 Fekanich D, Hunter D, Willett W *et al.*: Vitamin-D receptor genotype and risk of bone fracture in women. *Epidemiology* 9(5), 535–539 (1998).

82 Ioannidis JP, Ralston SH, Bennett ST *et al.*: Differential genetic effects of *ESR1* gene polymorphisms on osteoporosis outcomes. *JAMA* 292(17), 2105–2114 (2004).

83 Massart F, Marini F, Bianchi G *et al.*: Age-specific effects of estrogen receptors′ polymorphisms on the bone traits in healthy fertile women: the BONTURNO study. *Reprod. Biol. Endocrinol.* 7(32), 1–7 (2009).

84 Becherini L, Gennari L, Masi L *et al.*: Evidence of a linkage disequilibrium between polymorphisms in the human estrogen receptor α gene and their relationship to bone mass variation in postmenopausal Italian women. *Hum. Mol. Genet.* 9(13), 2043–2050 (2000).

85 Langdahl BL, Lokke E, Carstens M, Stenkjaer LL, Eriksen EF: A TA repeat polymorphism in the estrogen receptor gene is associated with osteoporotic fracture but polymorphism in the first exon and intron are not. *J. Bone Miner. Res.* 15(11), 2222–2230 (2000).

86 Mann V, Ralston SH: Meta-analysis of *COL1A1 Sp*1 polymorphism in relation to bone mineral density and osteoporotic fracture. *Bone*. 32(6), 711–717 (2003).

87 Ralston SH, Uitterlinden AG, Brandi ML *et al.*: Large-scale evidence for the effect of the *COLIA1 Sp*1 polymorphism on osteoporosis outcomes: the GENOMOS Study. *PLoS Med.* 3(5), E223 (2006).

88 Husted L, Harslof T, Gonzalez-Bofill N, Schmitz A, Carstens M, Stenkjaer L: Haplotypes of promoter and intron-1 polymorphisms in the *COLIA1* gene are associated with increased risk of osteoporosis. *Calcif. Tissue Int.* 84(2), 85–96 (2009).

89 Yazdanpanah N, Rivadeneira F, van Meurs JB *et al.*: The -1997 G/T and *Sp*1 polymorphisms in the collagen type I α1 (*COLIA1*) gene in relation to changes in femoral neck bone mineral density and the risk of fracture in the elderly: the Rotterdam study. *Calcif. Tissue Int.* 81(1), 18–25 (2007).

90 MacLaughlin E: Improving osteoporosis screening, risk assessment, diagnosis, and treatment initiation: role of the health-system pharmacist in closing the gap. *Am. J. Health Syst. Pharm.* 67(3), 4–8 (2010).

▪ **Gap between evidence and practice related to screening and treatment of osteoporosis, fractures, morbidity and mortality, and new approaches to risk assessment and treatment decisions.**

91 Lamberts S, Uitterlinden A: Genetic testing in clinical practice. *Annu. Rev. Med.* 60, 431–442 (2009).

▪ **Highlights thatin the practice of internal medicine, the current value of genetic testing in complex genetic diseases such as osteroporosis is limited.**

92 van Straaten T, Van Schaik R: Genetic techniques for pharmacogenetic analyses. *Curr. Pharm. Des.* 16(2), 231–237 (2010).

▪ **Potential of pharmacogenetic testing of individual patients to optimize drug treatment is expanding owing to exponential increase in DNA markers explaining and/or predicting response to drug therapy.**

93 Weinshilboum R: Inheritance and drug response. *N. Engl. J. Med.* 348(6), 529–537 (2003).

94 Lewiecki E, Bilezikian JP, Laster A *et al.*: 2009 Santa Fe bone symposium. *J. Clin. Densitom.* 13(1), 1–9 (2010).

95 Delmas PD, Rizzoli R, Cooper C, Reginster JY: Treatment of patients with postmenopausal osteoporosis is worthwhile. The position of the International Osteoporosis Foundation. *Osteoporos. Int.* 16(1), 1–5 (2005).

96 Nguyen D, Eisman J, Nguyen T: Anti-hip fracture efficacy of bisphosphonates: a Bayesian analysis of clinical trials. *J. Bone Miner. Res.* 21(2), 340–349 (2006).

97 Francis RM: Nonresponse to osteoporosis treatment. *J. Br. Menopause Soc.* 10(2), 76–80 (2004).

98 Nguyen TV, Eisman JA: Pharmacogenomics of osteoporosis: opportunities and challenges. *J. Musculoskelet. Neuronal Interact.* 6(1), 62–72 (2006).

99 Kennel KA, Drake MT: Adverse effects of bisphosphonates: implicactions for osteoporosis management. *Mayo Clin. Proc.* 84(7), 632–638 (2009).

100 Sarasquete ME, García-Sanz R, Marín L *et al.*: Bisphosphonate-related osteonecrosis of the jaw is associated with polymorphisms of the cytochrome P450 *CYP2C8* in multiple myeloma: a genome-wide single nucleotide polymorphism analysis. *Blood* 112(7), 2709–2712 (2008).

101 Palomba S, Numis FG, Mossetti G *et al.*: Effectiveness of alendronate treatment in postmenopausal women with osteoporosis: relationship with *Bsm*I Vitamin-D receptor genotypes. *Clin. Endocrinol. (Oxf).* 58(3), 365–371 (2003).

102 Palomba S, Orio F Jr, Russo T *et al.*: *Bsm*I vitamin-D receptor genotypes influence the efficacy of antiresorptive treatments in postmenopausal osteoporotic women. A 1-year multicenter, randomized and controlled trial. *Osteoporos. Int.* 16(8), 947–952 (2005).

103 Arko B, Prezelj J, Komel R, Kocijancic A, Marc J: No major effect of estrogen receptor β gene *Rsa*I polymorphism on bone mineral density and response to alendronate therapy in postmenopausal osteoporosis. *J. Steroid Biochem. Mol. Biol.* 81(2), 147–152 (2002).

104 Marc J, Prezelj J, Komel R, Kocijancic A: *VDR* genotype and response to etidronate therapy in late postmenopausal women. *Osteoporos. Int.* 10(4), 303–306 (1999)

105 Deng HW, Li J, Li JL *et al.*: Change of bone mass in postmenopausal Caucasian women with and without hormone replacement therapy is associated with vitamin-D receptor and estrogen receptor genotypes. *Hum. Genet.* 103(5), 576–585 (1998).

106 Tofteng CL, Jensen JE, Abrahamsen B, Odum L, Brot C: Two polymorphisms in the vitamin-D receptor gene association with bone mass and 5-year change in bone mass with or without hormone-replacement therapy in postmenopausal women: the Danish osteoporosis prevention study. *J. Bone Miner. Res.* 17(8), 1535–1544 (2002).

107 Rogers MJ: New insights into the molecular mechanisms of action of bisphosphonates. *Curr. Pharm. Des.* 9(32), 2643-2658 (2003).

108 Carbonell S, Masi L, Marini F *et al.*: Genetics and pharmacogenetics of osteoporosis. *J. Endocrinol. Invest.* 28(10), 2–7 (2005).

109 Marini F, Falchetti A, Silvestri S *et al.*: Modulatory effect of farnesyl pyrophosphate synthase (*FDPS*) rs2297480 polymorphism on the response to long-term amino-bisphosphonate treatment in postmenopausal osteoporosis. *Curr. Med. Res. Opin.* 24(9), 2609–2615 (2008).

110 van der Straaten T, van Schaik RH: Genetic techniques for pharmacogenetic analyses. *Curr. Pharm. Des.* 16(2), 231–237 (2010).

111 Suwazono Y, Kobayashi E, Uetani M *et al.*: G-protein β 3 subunit polymorphism C1429T and low-density lipoprotein receptor-related protein 5 polymorphism A1330V are risk factors for hypercholesterolemia in Japanese males – a prospective study over 5 years. *Metabolism* 55(6), 751–757 (2006).

112 Bojesen SE, Juul K, Schnohr P, Tybjærg-Hansen A, Nordestgaard BG: Platelet glycoprotein IIb/IIIa PlA2/PlA2 homozygosity associated with risk of ischemic cardiovascular disease and myocardial infarction in young men. *JACC* 42(4), 661–667 (2003).

113 Penna-Martinez M, Ramos-López E, Stern J *et al.*: Vitamin-D receptor polymorphisms in differentiated thyroid carcinoma. *Thyroid* 19(6), 623–628 (2009).

114 Morrison NA, George PM, Vaughan T, Tilyard MW, Frampton CM, Gilchrist NL: Vitamin-D receptor genotypes influence the success of calcitriol therapy for recurrent vertebral fracture in osteoporosis. *Pharmacogenet. Genomics* 15(2), 127–135 (2005).

115 Quevedo LI, Martínez BM, Castillo NM, Rivera FN: Vitamin-D receptor gene polymorphisms and risk of hip fracture in Chilean elderly women. *Rev. Med. Chil.* 136(4), 475–481 (2008).

116 Macdonald H, Mcguigan F, Stewart A *at al.*: Large-scale population-based study shows no evidence of association between common polymorphism of the *VDR* gene and BMD in British women. *J. Bone Miner. Res.* 21(1), 151–162 (2006).

117 Ashton KA, Proietto A, Otton G *et al.*: Estrogen-receptor polymorphisms and the risk of endometrial cancer. *BJOG* 116(8), 1053–1061 (2009).

118 Grant S, Reid D, Blake G, Herd R, Fogelman I, Ralston S: Reduced bone density and osteoporosis associated with a polymorphic *Sp*1 binding site in the collagen type Iα1 gene. *Nat. Genet.* 14(2), 203–205 (1996).

119 Jiang H, Lei SF, Xiao SM *et al.*: Association and linkage analysis of *COL1A1* and *AHSG* gene polymorphisms with femoral neck bone geometric parameters in both Caucasian and Chinese nuclear families. *Acta Pharmacol. Sin.* 28(3), 375–381(2007).

Websites

201 Clinican's Guide to prevention and treatment of osteoporosis. National Osteoporosis Foundation, Washington, DC, USA (2008) www.nof.org/professionals/Clinicians_Guide.htm

202 Uitterlinden A, Pols H: Manual Práctico de osteoporosis y enfermedades del metabolismo mineral: Genética de la Osteoporosis. ISBN: 84-88992-91-2. Cap 10, 49–54 (2004) www.medicrit.com/libros/OSTEOPOROSIS/49.pdf

203 FRAX® WHO fracture risk assessment tool (2009) www.shef.ac.uk/FRAX

204 Garvan–Dubbo Fracture Risk calculator www.fractureriskcalculator.com.au

205 European Medicines Agency: Pre-authorisation evaluation of medicines for human use: denosumab www.ema.europa.eu/pdfs/human/opinion/Prolia_77616809en.pdf

206 National Institute for Health and Clinical Excellence (NICE): Technology appraisal guidance 160: bisphosphonates (alendronate, etidronate, risedronate), raloxifene, strontium ranelate and teriparatide for the primary prevention of osteoporotic fragility fractures in postmenopausal women 92008) www.nice.org.uk/TA087guidance

207 National Institute for health and Clinical Excellence (NICE): Technology appraisal guidance 161: bisphosphonates (alendronate, etidronate, risedronate), raloxifene, strontium ranelate and teriparatide for the secondary prevention of osteoporotic fragility fractures in postmenopausal women (2008) www.nice.org.uk/TA087guidance

208 Pharmacogenomics Knowledge Base (PharmGKB) www.pharmgkb.org

209 HUGO nomenclature www.genenames.org